

Section II (Remarks)

A. Summary of Amendment to the Claims

By the present Amendment, claims 1, 2, 4 and 11 have been amended. As amended, the claims are supported by the specification and the original claims. Support for the amendment to claim 1 is found in claim 3, as originally filed. Claim 2 has been amended to correct a self-evident typographical error in the spelling of “gelatin.” Claim 4 has been amended to ensure proper dependency, in light of the cancellation of claim 3. Claim 11 has been amended to clarify the language therein. Specifically, the term “subsequent” has been removed and the phrase “characterized in that” has been replaced by “wherein.” No new matter has been added, as defined by 35 U.S.C. § 132.

By the present amendment, cancellation of claim 3 is requested, without prejudice.

Thus, upon entry of the amendments, claims 1, 2, and 4-13 will be pending and under examination.

B. Regarding the specification

In the Office Action mailed February 2, 2010, the examiner requested that use of the term “transwell” be capitalized and followed by the symbol indicating a registered trademark (®) each time the term appears in the specification. It is respectfully submitted, with the rationale for such submission detailed below, that the term “transwell” is used generically by those in the art, not necessarily in reference to the trademarked product “TRANSWELL[®]” (U.S. Reg. No. 1,415,743).

C. Rejection Under 35 U.S.C. §, 112, Second paragraph

In the Office Action mailed February 2, 2010, the examiner rejected claims 3, 7 and 13 as indefinite, with regard to the phrase “polarized” in claim 3 and “transwell” in claims 7 and 13. Applicants respectfully disagree.

It is well established, as set forth in MPEP §2173.02, that definiteness of claim language must be analyzed, not in a vacuum, but in light of:

- (A) The content of the particular application disclosure;
- (B) The teachings of the prior art; and
- (C) The claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made.

The examiner's attention is respectfully drawn to Section II above, where claim 3 has been cancelled. However, as amended, claim 1 contains recitation of "polarized." Accordingly, the rejection is addressed below as applicable to amended claim 1.

With regard to the term "polarized" recited in claim 1, while such term can have different meanings in cell biology, there is no lack of clarity with regard to the intended meaning of this term in the present application. It is widely known in the field of the invention that cellular polarization (cell polarity or the like) essentially refers to asymmetry in the organization or development of cells, in concrete, cells showing distinct apical and basolateral surfaces. The apical membrane of a polarized cell is the surface of the plasma membrane that faces the lumen, whereas the basolateral membrane of a polarized cell is the surface of the plasma membrane that forms its basal and lateral surfaces; it faces towards the interstitium, and away from the lumen.

For example, cellular polarization is best established in epithelium. The plasma membrane of epithelial cells is divided into two domains: an apical domain facing the external milieu and a basolateral domain in contact with the internal milieu and the blood supply. These plasma membrane domains have different lipid and protein compositions (Simons et al. 1988; van Meer et al. 1988). Epithelial cells have tight junctions, which separate the apical and basolateral membranes from each other. The general features defining cell polarity have been described, for example, in cultured (MDCK, Caco-2) and primary epithelial cells (Handler 1989, Rodriguez-Boulan et al. 1992). Many of the functions of epithelial, neuronal and certain other cells of the immunosystem are maintained by the characteristically polarized phenotype of these cells (Cereijido et al. 1989, Craig et al. 1992, Knust 1994, Nelson 1993, Rodriguez-Boulan et al. 1992).

In addition to the above-cited references the following list of references (*see* Exhibit A) is provided, each of which relates to polarized cultures. These documents demonstrate knowledge of those of skill in the art with regard to use of the term "polarized," as used in amended claim 1:

- Fang HW, Fang SB, Chiang Chiau JS, Yeung CY, Chan WT, Jiang CB, Cheng ML, Lee HC, "Inhibitory effects of *Lactobacillus casei subsp. rhamnosus* on *Salmonella* lipopolysaccharide-induced inflammation and epithelial barrier dysfunction in a co-culture model using Caco-2/peripheral blood mononuclear cells;" J. Med. Microbiol. 2010 May;59 (Pt. 5):573-9.
- Halbleib JM, Sääf AM, Brown PO, Nelson WJ; "Transcriptional modulation of genes encoding structural characteristics of differentiating enterocytes during development of a polarized epithelium *in vitro*;" Mol. Biol. Cell. 2007 Nov;18(11):4261-78. Epub 2007 Aug 15.
- Wang C, Qiu Z. Sheng Wu Yi Xue Gong Cheng Xue Za Zhi; "Development of researches in Caco-2 cell model;" 2005 Jun;22(3):633-6, 644.
- Neunlist M, Tounsi F, Oreschkova T, Denis M, Leborgne J, Laboisse CL, Galmiche JP, Jarry A.; "Human ENS regulates the intestinal epithelial barrier permeability and a tight junction-associated protein ZO-1 via VIPergic pathways;" Am. J. Physiol. Gastrointest. Liver Physiol. 2003 Nov;285(5):G1028-36.
- Meunier V, Bourrié M, Berger Y, Fabre G.; "The human intestinal epithelial cell line Caco-2; pharmacological and pharmacokinetic applications;" Cell Biol. Toxicol. 1995 Aug;11(3-4):187-94. Review.
- Wells CL, Jechorek RP, Olmsted SB, Erlandsen SL; "Effect of LPS on epithelial integrity and bacterial uptake in the polarized human enterocyte-like cell line Caco-2;" Circ. Shock. 1993 Aug;40(4):276-88.

In view of the above, it is respectfully submitted that use of the term "polarized" in amended claim 1 is clear and satisfies the written description requirement of 35 U.S.C. §112, second paragraph. Withdrawal of the rejection is respectfully requested.

With regard to use of the term "transwell" in claims 7 and 13, cell cultures have become a key research and development issue at all levels of research. At present, it is mandatory to develop environmental systems resembling body physiological conditions in order to allow the growth of specialized cell types. Consequently, permeable supports or inserts with microporous membranes, commonly known as transwells, have become standard tools for culturing of cells. The use of these supports has enabled a high level of improvement to be achieved in culturing polarized cells, since permeable membrane filters permit cells to feed basolaterally and carry out metabolic activities in a similar way to that occurring under physiological conditions.

In the 1950's, filters appeared as a solution for cell culture. Filters have been updated over the years and nowadays, permeable supports are part of multiple cell culture protocols under many different experimental conditions. Filters are now recognized as providing significant advantages over solid, impermeable cell growth substrates.

Due to the fact that permeable supports allow access to both, apical and basolateral areas, cellular functions such as transport, adsorption, and secretion can also be studied.

It has been demonstrated that permeable asymmetric supports are indispensable in all laboratories investigating various aspects of cell biology.

Therefore, it is clear that the term “transwell” is commonly used in the art to identify a type of permeable support with a microporous membrane, resembling as closely as possible the *in vivo* environment. This particular type of permeable support provides independent access to both sides of a monolayer, thus giving researchers a versatile tool to study transport and other metabolic activities *in vitro*. Standard transwell inserts are available in three membrane materials [polycarbonate (PC), polyester (PET) and collagen-coated polytetrafluoroethylene (PTFE)] and in different pore sizes (0.4-8 µm) and different insert diameters (6.5, 12, 24, and 75mm).

Transwell-type supports allow some cell types (*e.g.* epithelial cells) to be grown in a polarized state under more natural conditions. For example, when cultured in this format, the caco-2 cells differentiate to form a polarized epithelial cell monolayer that provides a physical and biochemical barrier to the passage of ions and small molecules. The Caco-2 monolayer is widely used across the pharmaceutical industry as an *in vitro* model of the human small intestinal mucosa to predict the absorption of orally administered drugs.

The following list of references (*see* Exhibit B) is provided, each of which utilize the term “transwell” in a generic manner. These documents demonstrate that, to those of skill in the art, “transwell” is used to generally refer to a type of permeable support with a microporous membrane:

- Babich H, Sinensky MC; “Indirect cytotoxicity of dental materials: a study with Transwell inserts and the neutral red uptake assay;” *Altern Lab Anim.* 2001 Jan-Feb; 29(1):9-13.
- Wei L, Debets R, Hegmans JJ, Benner R, Prens EP; “IL-1 beta and IFN-gamma induce the regenerative epidermal phenotype of psoriasis in the transwell skin organ culture system. IFN-gamma upregulates the expression of keratin 17 and keratinocyte transglutaminase via endogenous IL-1 production;” *J Pathol.* 1999 Feb;187(3):358-64.
- Connolly L, Maxwell P.; “Image analysis of Transwell assays in the assessment of invasion by malignant cell lines;” *Br J Biomed Sci.* 2002; 59(1):11-4.

- Asami N, Germeraad WT, Fujimoto S, Nagai S, Izumi T, Katsura Y; "Gene transduction into murine primitive hematopoietic cells with 2-gene retroviral vectors using a Transwell coculture system;" *Eur J Haematol.* 1996 Oct; 57(4):278-85.

Additionally, the examiner's attention is further drawn to the Curatolo reference, cited in the February 2, 2010 Office Action, which also references transwell supports.

In view of the above, it is respectfully submitted that use of the term "transwell" in claims 7 and 13 is clear and satisfies the written description requirement of 35 U.S.C. §112, second paragraph. Withdrawal of the rejection is respectfully requested.

Additionally in the February 2, 2010 Office Action, claim 11 was rejected under 35 U.S.C. §112 as indefinite for use of the phrase "the subsequent incubation." As amended, claim 11 no longer contains the term "the subsequent incubation." The rejection is therefore moot and withdrawal is respectfully requested.

D. Rejection Under 35 U.S.C. §. 102

In the February 2, 2010 Office Action, the examiner rejected claims 1, 6 and 12 under 35 U.S.C. §102(b) as anticipated by European Patent Application No. EP 0702081 (hereinafter "Morota et al.") Applicants respectfully traverse the rejection.

In particular, the examiner contended that Morota et al. teaches the claimed methods. Applicants respectfully direct the examiner's attention to page 2, col. 2, lines 5-11 of Morota et al., which states in relevant part: "...it is an object of the invention to provide a matrix for tissue culture and a method for culturing tissue which make it possible to obtain tissue culture having a desired three-dimensional structure..." (emphasis added). Such a three dimensional structure is contrasted with "...conventional tissue models and artificial organs [that] do not have a desired three-dimensional structure..." (Morota et al., page 2, col. 1, lines 55-56.)

Therefore Morota et al. describe a process for storing and fixing a culture tissue having a three-dimensional structure, in particular a cultured skin model, by adding a gelatin solution dissolved in a culture medium at a concentration between 1 and 10%.

One particular feature of the system described in the Morota et al. is that the gelatin solution does not coat the cultured tissue, but it surrounds the sponge containing the tissue (see page 4,

col. 5, line 15). The cultured skin model has a two-layer structure of dermis and epidermis. Said dermis layer is cultured in a first sponge while the epidermis layer is inoculated on a second sponge (support), this second sponge being subsequently degraded so that epidermis-forming cells are located upon the dermis layer. When the gelatin solution is added to this tissue, the epidermis layer (inoculated in the second sponge) is in contact with gas phase/atmosphere and the dermis layer (cultured in the first sponge) would be partially immersed in the gelatin solution so that nutrients of the culture medium could reach and feed said layer. According to Morota et al., this feature avoids problems existing in skin cultures, such as swelling and death of cells, in particular when epidermis does not contact with the atmosphere and when the dermis does not contact with nutrient supply [see page 4, col. 5, lines 27-32].

By contrast, applicants' claimed invention, as recited in claim 1, differs from the method of Morota et al as follows:

- Claim 1(a): the claimed method is applied over organized, differentiated, polarized and functionally active two-dimensional cell cultures where the cells are in a suitable functional state;
- Claims 1(a)-(c): the claimed method requires the specific combination of: gelatin concentration of 1-5%, solidifying at 15-25°C, and storing at 15-25°C for a period of 96 hours;
- Claim 1(a): the gelatin coats the two-dimensional cell culture.

None of the above limitations of applicants' claimed method are described in Morota et al. Anticipation of a claim requires the disclosure in a single prior art reference of each element of the claim under consideration. (*Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987.)) As such, claim 1 is not anticipated by Morota et al. Claims 6 and 12 are patentable for the same reasons advanced above in support of the patentability of claim 1.

Since Morota et al. does not describe a method as set forth in claims 1, 6 and 12, Morota et al. does not anticipate the claimed invention. Accordingly, withdrawal of the rejection of claims 1, 6 and 12 under 35 U.S.C. § 102(b) as being anticipated by Morota et al., is respectfully requested.

E. Rejection Under 35 U.S.C. §, 103

In the February 2, 2010 Office Action, claims 1 to 13 were rejected under 35 U.S.C. §103(a) as being unpatentable over European Patent Application No. EP 1127580 (hereinafter “Curatolo et al.” and Morota et al. or over International Patent Application Publication No. WO 01/66783 (hereinafter “Lee et al.” in view of Morota et al. Applicants traverse the rejections.

A *prima facie* case of obviousness based on the rationale that the invention could have been achieved by “combining prior art elements according to known methods to yield predictable results” is supportable by a showing that “all the claimed elements were known in the prior art and one skilled in the art could have combined the elements as claimed by known methods with no change in their respective functions, and the combination yielded nothing more than predictable results to one of ordinary skill in the art.” See MPEP §2143, citing the U.S. Supreme Court decision in *KSR International Co. v. Teleflex Inc.*, 550 U.S. 398, 82 USPQ2d 1385 (2007). Neither of the cited combinations of Curatolo et al. in view of Morota et al. or Lee et al. in view of Morota et al. shows that all elements of applicants’ claimed invention were known in the prior art.

As the Examiner acknowledges on page 5 of the Office Action, neither Curatolo et al. nor Lee et al. expressly teach a method of storing by coating the cell culture support with gelatin, solidifying the gelatin, storing the cells, or liquefying the gelatin. The passages of Curatolo et al. and Lee et al. indicated by the Examiner merely describe cell culture techniques as *in vitro* models for assessing the azithromycin transport across Caco-2 Cell Monolayers (Curatolo et al.) or the inhibitory activity against VEGF-Induced HUVEC proliferation (Lee et al.). Both of these references are related to the growth of cells and subsequent analysis, but neither reference, in any way discloses or suggests a method of storing and/or transporting cultures so that the cell culture maintains its functional state. Nor does either of the cited primary references, Curatolo et al. or Lee et al., describe the specific steps included in the method recited in independent claim 1.

Contrary to the Examiner’s assertion, Morota et al. does not cure the deficiencies of either of Curatolo et al. or Lee et al. It is respectfully submitted that Morota et al. does not relate to the same problem and does not suggest the solution as recited in independent claim 1. Applicants’ claimed invention provides a solution to the problem of maintaining the functional state (defined in the specification at p. 8, lines 1-3) of the specific cell cultures defined in claim 1, so that they

may be used in an assay within a sufficient timeframe. By contrast, Morota et al. does not relate to *in vitro* models, nor to polarized cells, and does not suggest the surprising result that organized, differentiated polarized two-dimensional cells cultures could be stored and transported by the methods of applicants' claimed invention and that the cultures will maintain their functionalization for *in vitro* tests after storage, as shown in the examples.

The methods recited in applicants' claimed invention unexpectedly make the maintenance of the physiological properties of two-dimensional cell cultures possible, in addition to the protection of mechanical properties during the storing and transport of cell cultures. This allows use of the culture, and its functional properties once the gelatin is removed, up to 9 days thereafter (*see* Specification, example 1, p. 12, lines 1-11). Indeed, the fixing method defined in claim 1 allows the immobilization of a Caco-2 cell culture for up to 4 days at room temperature without affecting its functional barrier state (established by measuring Trans Epithelial Electric Resistance, TEER) and once the gelatin has been removed to perform barrier permeability assays. Additionally, it has been shown for other two-dimensional cell types that the functionality is maintained by adhesion, migration and invasion assays (*see* Specification, example 2 and last paragraph of the description).

These advantages are particularly relevant for polarized, differentiated two-dimensional cell cultures due to their complexity and their temporal limitations, since once a functional culture is obtained, it is particularly difficult to maintain its functional properties unimpaired and to allow passage of a sufficient time for making *in vitro* assays without losing the functionality of the cell culture (*see* Specification, p. 7, lines 7-34).

As discussed above with respect to the rejection under 35 U.S.C. §112, polarization is a term that is well known to one of skill in the art of cell cultures. The term relates to cells that exhibit structural and functional asymmetry between apical and basolateral surfaces. The cells have plasma membranes specialized to receive specific chemical signals originating from the external (or apical surface) and internal (or basolateral surface) environments of the organism. This feature makes this type of cell cultures especially complex since they require continuous manipulation to maintain the properties that make them a suitable model to imitate the natural barriers (veins, intestine, etc.) of the organism. For example, Caco-2 cells (polarized cells) require 21 days to reach the state of differentiation which allows many of the properties of

intestinal mucous to be reproduced, and their use is prolonged only during a window of approximately 3 to 5 days (*see* Specification, p. 1-2).

Both Curatolo et al. in view of Morota et al. and Lee et al. in view of Morota et al. fail to provide any derivative basis for the claimed invention and, additionally, there would have been no logical reason for one of skill in the art to combine such references. Accordingly, no basis of *prima facie* obviousness of the claimed invention is presented by such cited references.

In looking for a solution to the problem of creating a polarized, differentiated two-dimensional cell culture and storage and transporting of the same, one of skill in the art would not have considered the teaching of Morota et al.

The fixing method described in Morota et al. is used with the aim of avoiding death and mechanical impairment in a three-dimensional cultured tissue. Morota et al. does not analyze if dermis and epidermis cells maintain their functionality or not. In Morota et al., the culture model is only visually observed by microscopy (*see* p. 5, col. 8, lines 19-34), which allows determining of whether a mechanical impairment has occurred or if the cells are not dead. However, in Morota et al. there is no assessment of whether the cellular model is functionally active.

Furthermore, Morota et al. does not relate to *in vitro* models, nor to polarized cells, and does not suggest the surprising result that, organized, differentiated polarized two-dimensional cell cultures could be stored and transported by the method of applicants' claimed invention and that the cultures will keep their functionalization for *in vitro* tests after storage, as shown in the examples.

Additionally, in the fixing system described in Morota et al., there are no cells directly coated by gelatin. In Morota et al. the epidermis cells formed upon dermis cells are not immersed in the gelatin solution (the epidermis layer is kept in contact with the atmosphere). It is only the first sponge, wherein the dermis cells are cultured, that is surrounded by the gelatin solution. In fact, Morota et al. teaches that epidermis cells must not be coated with the gelatin solution in order to avoid their death. Thus, Morota et al. teaches the skilled person that specific tissues have specific needs, teaching away from taking away the sponge support and directly coating any cell culture with gelatin.

In considering a reference for its effect on patentability, the reference is required to be considered in its entirety, including portions that teach away from the invention under consideration. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984); MPEP § 2141.02. Morota et al. teach one of skill in the art away from completely coating an organized two-dimensional cell culture with gelatin, since, according to the teaching of the Morota publication, this could seriously affect the cell functionality.

Furthermore, the support described in Morota et al. consists of a combination of two sponges with different physical and chemical features (p. 3, col. 4, lines 20-24), which allows, on the one hand, the three-dimensional proliferation of fibroblasts (dermis) within the structure of the first sponge, and on the other hand, the proliferation of the keratinocytes (epidermis) on the surface of the second sponge. It would not be obvious for the skilled person that the method of Morota et al. would equally work in absence of the specific sponges.

The asymmetric support used in applicants' claimed invention (*see* Specification, p. 3, para. 13) makes it possible for the defined cells to get the functional state (polarization) prior to coating with gelatin, as stated in example 1, part 2.

In order to arrive at applicants' claim 1 in view of secondary reference Morota et al., one of skill in the art would have to disregard the use of sponge supports in Morota et al., disregard the fact that Morota et al. is directed to a specific type of tissue with special needs (epidermis should be in contact with air, three dimensional structure has to be maintained), and disregard the fact that the present claim 1 is directed to a very different type of cell culture: differentiated, polarized, functionally active two-dimensional cell cultures.

Based on the foregoing, Curatolo et al. in view of Morota et al. and Lee et al. in view of Morota et al. fail to provide any logical basis for the methods and kits recited in claims 1, 2, and 4-13.

Specifically, the cited combinations of references do not address the problems related to the preservation of functional properties during storing and transport of organized two-dimensional cell cultures, nor the possibility of using the cultures after removing the gelatin, for a reasonably long period of time, without losing their functional properties. In addition, the cited

combinations of references are also silent about the carefully and specifically chosen combination of parameters (concentration, temperatures, time) recited in applicant's method of claim 1.

Neither Curatolo et al. in view of Morota et al., nor and Lee et al. in view of Morota et al. render the claimed invention obvious. Accordingly, withdrawal of the rejection of claims 1, 2, and 4-13 under 35 U.S.C. § 103(a) as being obvious over Curatolo et al. in view of Morota et al. and Lee et al. in view of Morota et al. is respectfully requested.

CONCLUSION

Based on the foregoing, all of applicants' pending claims 1, 2, and 4-13 are patentably distinguished over the art, and in form and condition for allowance. The examiner is requested to favorably consider the foregoing and to responsively issue a Notice of Allowance.

This responds to the February 2, 2010 Office Action in the above-identified application. The time for responding to the February 2, 2010 Office Action without extension was set at three months, or May 2, 2010. Applicants hereby request a three month extension of time under 37 CFR § 1.136 to extend the deadline for response to and including August 2, 2010. Payment of the extension fee of \$555.00 specified in 37 C.F.R. § 1.17(a)(3), as applicable to small entity, is being made by on-line credit card authorization at the time of EFS submission of this Response. Should any additional fees be required or an overpayment of fees made, please debit or credit our Deposit Account No. 08-3284, as necessary.

If any issues require further resolution, the examiner is requested to contact the undersigned attorneys at (919) 419-9350 to discuss same.

Respectfully submitted,

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Enclosures:
Exhibit A
Exhibit B

<p>The USPTO is hereby authorized to charge any deficiency or credit any overpayment of fees properly payable for this document to Deposit Account No. 08-3284</p>
